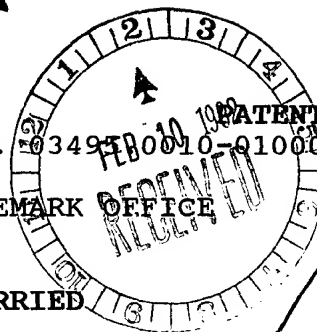




Attorney Docket No. 3495-1000-01000



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Application of:

MARC ALIZON et al.

Serial No.: 158,652

Filed: February 22, 1988

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Group Art Unit: 1805

Examiner: J. Railey

For: CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA
OF LYMPHADENOPATHY ASSOCIATED VIRUS (LAV) AND
PROTEINS ENCODED BY SAID LAV GENOMIC RNA

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

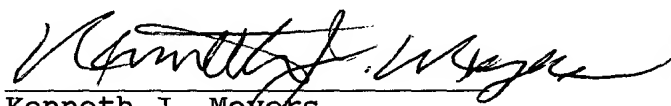
Sir:

SUBMISSION OF COPY OF PRIORITY DOCUMENT

Further to the Examiner's telephone request, enclosed
herewith is a copy of United Kingdom Priority Document
No. 8429099.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER

By: 
Kenneth J. Meyers
Reg. No. 25,146

Dated: February 6, 1992

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25 Southampton Buildings
London, WC2A 1AY

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REQUEST FOR GRANT OF A PATENT

THE GRANT OF A PATENT IS REQUESTED BY THE UNDERSIGNED ON THE BASIS OF THE PRESENT APPLICATION

1 Agent's Reference JJD/EEF/26804

11 Title of Invention CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF
LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS
ENCODED BY SAID LAV GENOMIC RNA.

111 Applicant or Applicants (See note 2)

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XXXXXXXXXXXXXXXXXXXXXXXXXXXX

(b) A statement on Patents Form No. 7/77 is/will be furnished

V Name of Agent (if any) (See note 4)

Reddie & Grose

ADP CODE NO

VI Address for Service (See note 5)

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VII Declaration of Priority (See note 6)

Country

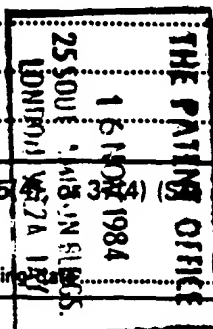
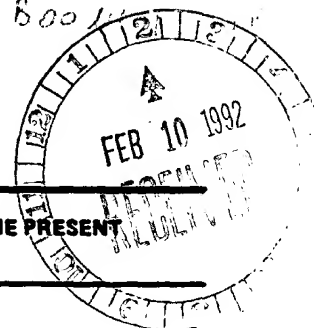
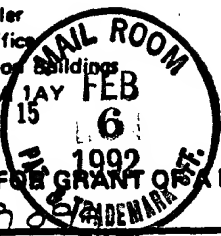
Filing date

File number

VIII The Application claims an earlier date under Section 8(3), 12(6), 15(4), or 35(4) (See note 7)

Section No.

Earlier application or patent number and filing date



IX Check List (To be filled in by applicant or agent)

- | | |
|---|---|
| <p>A The application contains the following number of sheet(s)</p> <p>1 Request 1 Sheet(s)</p> <p>2 Description 17 Sheet(s)</p> <p>3 Claim(s) 2 Sheet(s)</p> <p>4 Drawing(s) 26 Sheet(s)</p> <p>5 Abstract 0 Sheet(s)</p> | <p>B The application as filed is accompanied by:-</p> <p>1 Priority document No</p> <p>2 Translation of priority document No</p> <p>3 Request for Search No</p> <p>4 Statement of Inventorship and Right to Apply No</p> <p>5</p> |
|---|---|

X It is suggested that Figure No 1 of the drawings (if any) should accompany the abstract when published.

XI Signature (See note 8)


Reddie & Grose, Agents for the Applicant(s)

NOTES:

1. This form, when completed, should be brought or sent to the Patent Office together with the prescribed fee and two copies of the description of the invention, and of any drawings.
2. Enter the name and address of each applicant. Names of individuals should be indicated in full and the surname or family name should be underlined. The names of all partners in a firm must be given in full. Bodies corporate should be designated by their corporate name and the country of incorporation and, where appropriate, the state of incorporation within that country should be entered where provided. Full corporate details, eg "a corporation organised and existing under the laws of the State of Delaware, United States of America," trading styles, eg "trading as xyz company", nationality, and former names, eg "formerly [known as] ABC Ltd." are not required and should not be given. Also enter applicant(s) ADP Code No. (if known).
3. Where the applicant or applicants is/are the sole inventor or the joint inventors, the declaration (a) to that effect at IV should be completed, and the alternative statement (b) deleted. If, however, this is not the case the declaration (a) should be struck out and a statement will then be required to be filed upon Patent Form No 7/77.
4. If the applicant has appointed an agent to act on his behalf, the agent's name and the address of his place of business should be indicated in the spaces available at V and VI. Also insert agent's ADP Code No. (if known) in the box provided.
5. An address for service in the United Kingdom to which all documents may be sent must be stated at VI. It is recommended that a telephone number be provided if an agent is not appointed.
6. The declaration of priority at VII should state the date of the previous filing and the country in which it was made and indicate the file number, if available.
7. When an application is made by virtue of section 8(3), 12(6), 15(4), or 37(4) the appropriate section should be identified at VIII and the number of the earlier application or any patent granted thereon identified.
8. Attention is directed to rules 90 and 106 of the Patent Rules 1982.
9. Attention of applicants is drawn to the desirability of avoiding publication of inventions relating to any article, material or device intended or adapted for use in war (Official Secrets Acts, 1911 and 1920). In addition after an application for a patent has been filed at the Patent Office the comptroller will consider whether publication or communication of the invention should be prohibited or restricted under section 22 of the Act and will inform the applicant if such prohibition is necessary.
10. Applicants resident in the United Kingdom are also reminded that, under the provisions of section 23 applications may not be filed abroad without written permission or unless an application has been filed not less than six weeks previously in the United Kingdom for a patent for the same invention and no direction prohibiting publication or communication has been given or any such direction has been received.

Cloned DNA sequences related to the genomic RNA of lymphadenopathy-associated-virus (LAV) and proteins encoded by said LAV genomic RNA

The invention relates to cloned DNA sequences
5 indistinguishable from genomic RNA and DNA of lymphadenopathy-associated virus (LAV), a process for their preparation and their uses. It relates more particularly to stable probes including a DNA sequence which can be used for the detection of the LAV virus or related viruses
10 or DNA proviruses in any medium, particularly biological samples containing any of them. The invention also relates to polypeptides, whether glycosylated or not, encoded by said DNA sequences.

Lymphadenopathy-associated virus (LAV) is a human
15 retrovirus first isolated from the lymph node of a homosexual patient with lymphadenopathy syndrome, frequently a prodrome or a benign form of acquired immune deficiency syndrome (AIDS). Subsequently other LAV isolates have been recovered from patients with AIDS or pre-AIDS. All available data are consistent with the virus being the causative
20 agent of AIDS.

A method for cloning such DNA sequences has already been disclosed in British Patent Application Nr.
84 23859 filed on September 19, 1984. Reference is hereafter
25 made to that application as concerns subject matter in common with the further improvements to the invention disclosed herein.

The present invention aims at providing additional new means which should not only also be useful for the
30 detection of LAV or related viruses (hereafter more generally referred to as "LAV viruses"), but also have more versatility, particularly in detecting specific parts of the genomic DNA of said viruses whose expression products are not always directly detectable by immunological
35 methods.

The present invention further aims at providing

polypeptides containing sequences in common with polypeptides encoded by the LAV genomic RNA. It relates even more particularly to polypeptides comprising antigenic determinants included in the proteins encoded and expressed by the LAV genome occurring in nature. An additional object of the invention is to further provide means for the detection of proteins related to LAV virus, particularly for the diagnosis of AIDS or pre-AIDS or, to the contrary, for the detection of antibodies against the LAV virus or proteins related therewith, particularly in patients afflicted with AIDS or pre-AIDS or more generally in asymptomatic carriers and in blood-related products. Finally the invention also aims at providing immunogenic polypeptides, and more particularly protective polypeptides for use in the preparation of vaccine compositions against AIDS or related syndroms.

The present invention relates to additional DNA fragments, hybridizable with the genomic RNA of LAV as they will be disclosed hereafter, as well as with additional cDNA variants corresponding to the whole genomes of LAV viruses. It further relates to DNA recombinants containing said DNAs or cDNA fragments.

The invention relates more particularly to a cDNA variant corresponding to the whole of LAV retroviral genomes, which is characterized by a series of restriction sites in the order hereafter (from the 5' end to the 3' end).

The coordinates of the successive sites of the whole LAV genome (restriction map) are indicated hereafter too, with respect to the Hind III site (selected as of coordinate 1) which is located in the R region. The coordinates are estimated with an accuracy of ± 200 bp :

	Hind III	0
	Sac I	50
35	Hind III	520
	Pst I	800
	Hind III	1 100

	Bgl II	1 500
	Kpn I	3 500
	Kpn I	3 900
	Eco RI	4 100
5	Eco RI	5 300
	Sal I	5 500
	Kpn I	6 100
	Bgl II	6 500
	Bgl II	7 600
10	Hind III	7 850
	Bam HI	8 150
	Xho I	8 600
	Kpn I	8 700
	Bgl II	8 750
15	Bgl II	9 150
	Sac I	9 200
	Hind III	9 250

Another DNA variant according to this invention optionally contains an additional Hind III approximately
20 at the 5 550 coordinate.

Reference is further made to fig. 1 which shows a more detailed restriction map of said whole-DNA (λJ19).

An even more detailed nucleotide sequence of a preferred DNA according to the invention is shown in fig.
25 4-12 hereafter.

The invention further relates to other preferred DNA fragments which will be referred to hereafter.

Additional features of the invention will appear in the course of the non-limitative disclosure of additional features of preferred DNAs of the invention, as well
30 as of preferred polypeptides according to the invention. Reference will further be had to the drawings in which :
- fig. 1 is the restriction map of a complete LAV genome (clone λJ19) ;

35 - figs. 2 and 3 show diagrammatically parts of the three

possible reading phases of LAV genomic RNA, including the open reading frames (ORF) apparent in each of said reading phases :

- figs. 4-12 show the successive nucleotidic sequences of a complete LAV genome. The possible peptide sequences in relation to the three possible reading phases related to the nucleotidic sequences shown are also indicated ;
- figs. 13-18 reiterate the sequence of part of the LAV genome containing the genes coding for the envelope proteins, with particular boxed peptidic sequences which corresponds to groups which normally carry glycosyl groups.

The sequencing and determination of sites of particular interest was carried out on a phage recombinant corresponding to AJ19 disclosed in the abovesaid British Patent application Nr. 84 23659. A method for preparing it is disclosed in that application.

The whole recombinant phage DNA of clone AJ19 (disclosed in the earlier application) was sonicated according to the protocol of DEININGER (1983), Analytical Biochem. 129, 216. the DNA was repaired by a Klenow reaction for 12 hours at 16°C. The DNA was electrophoresed through 0.8 % agarose gel and DNA in the size range of 300-600 bp was cut out and electroeluted and precipitated. Resuspended DNA (in 10 mM Tris, pH 8 ; 0.1 mM EDTA) was ligated into M13mp8 RF DNA (cut by the restriction enzyme SmaI and subsequently alkaline phosphated), using T4 DNA- and RNA-ligases (Maniatis T et al (1982) - Molecular cloning - Cold Spring Harbor Laboratory). An *E. coli* strain designated as TGI was used for further study. This strain has the following genotype :

Alac pro, supE, thi.F'traD36, proA8, lacI^q, ZAM15,r⁻

This *E. coli* TGI strain has the peculiarity of enabling recombinants to be recognized easily. The blue colour of the cells transfected with plasmids which did

not recombine with a fragment of LAV DNA is not modified. To the contrary cells transfected by a recombinant plasmid containing a LAV DNA fragment yield white colonies. The technique which was used is disclosed in Gene (1983), 26.

5 101.

This strain was transformed with the ligation mix using the Hanahan method (Hanahan D (1983) J. Mol. Biol. 168, 557). Cells were plated out on tryptone-agarose plate with IPTG and X-gal in soft agarose. White plaques were
10 either picked and screened or screened directly in situ using nitrocellulose filters. Their DNAs were hybridized with nick-translated DNA inserts of pUC18 Hind III subclones of AJ19. this permitted the isolation of the plasmids or subclones of λ which are identified in the
15 table hereafter. In relation to this table it should also be noted that the designation of each plasmid is followed by the deposition number of a cell culture of E. coli TGI containing the corresponding plasmid at the "Collection Nationale des Cultures de Micro-organismes" (C.N.C.M.) of
20 the Pasteur Institute in Paris, France. A non-transformed TGI cell line was also deposited at the C.N.C.M. under Nr. I-364. All these deposits took place on November 15, 1984. The sizes of the corresponding inserts derived from the LAV genome have also been indicated.

25



TABLE

Essential features of the recombinant plasmids

5	- pJ19 - 1 plasmid	(I-365)	0.5 kb
	Hind III - Sac I - Hind III		
	- pJ19 - 17 plasmid	(I-367)	0.6 kb
10	Hind III - Pst I - Hind III		
	- pJ19 - 6 plasmid	(I-368)	1.5 kb
15	Hind III (5')		
	Sam HI		
	Xho I		
	Kpn I		
	Bgl II		
20	Sac I (3')		
	Hind III		
	- pJ19-13 plasmid	(I-368)	6.7 kb
25	Hind III (5')		
	Bgl II		
	Kpn I		
	Kpn I		
	Eco RI		
30	Eco RI		
	Sal I		
	Kpn I		
	Bgl II		
	Bgl II		
35	Hind III (3')		

Positively hybridizing M13 phage plates were grown up for 5 hours and the single-stranded DNAs were extracted.

M13mp8 subclones of λ J19 DNAs were sequenced according to the dideoxy method and technology devised by Sanger et al (Sanger et al (1977), Proc. Natl. Acad. Sci. USA, 74, 5483 and M13 cloning and sequencing handbook, AMERSHAM (1983). the 17-mer oligonucleotide primer α -³⁵SdATP (400Ci/mmol, AMERSHAM), and 0.5X-5X buffer gradient gels (Biggen M.D. et al (1983, Proc. Natl. Acad. Sci. USA, 50, 3963) were used. Gels were read and put into the computer under the programs of Staden (Staden R. (1982), Nucl. Acids Res. 10, 4731). All the appropriate references and methods can be found in the AMERSHAM M13 cloning and sequencing handbook.

The complete sequence of λ J19 was deduced from the experiments as further disclosed hereafter.

Figs. 4-12 provide the DNA nucleotide sequence of the complete genome of LAV. The numbering of the nucleotides starts from a left most Hind III restriction site (5'AAG...) of the restriction map. The numbering occurs in tens whereby the last zero number of each of the numbers occurring on the drawings is located just below the nucleotide corresponding to the nucleotides designated. I.e. the nucleotide at position 10 is T, the nucleotide at position 20 is C, etc..

Above each of the lines of the successive nucleotidic sequences there are provided three lines of single letters corresponding to the aminoacid sequence deduced from the DNA sequence (using the genetic code) for each at the three reading phases, whereby said single letters have the following meanings.

A : alanine
R : arginine
K : lysine
H : histidine
C : cysteine

M : méthionine
 W : tryptophan
 F : phenylalanine
 Y : tyrosine
 5 L : leucine
 V : valine
 I : isoleucine
 G : glycine
 T : thréonine
 10 S : serine
 E : glutamic acid
 D : Aspartic acid
 N : asparagine
 Q : glutamine
 15 P : proline.

The asterik signs "*" correspond to stop codons (i.e. TAA, TAG and TGA).

Starting above the first line of the DNA nucleotidic sequence of fig. 4 the three reading phases are respectively marked "1", "2", "3", on the left handside of the drawing. The same relative presentation of the three theoretical reading phases is then used all over the successive lines of the LAV nucleotidic sequence.

Figs. 2 and 3 provide a diagrammatized representation of the lengths of the successive open reading frames corresponding to the successive reading phases (also referred to by numbers "1", "2" and "3" appearing in the left handside part of fig. 2). The relative positions of these open reading frames (ORF) with respect to the nucleotidic structure of the LAV genome is referred to by the scale of numbers representative of the respective positions of the corresponding nucleotides in the DNA sequence. The vertical bars correspond to the positions of the corresponding stop codons.

35 1) The "gag gene" (or ORF-gag)

The "gag gene" codes for core proteins.

Particularly it appears that a genomic fragment (ORF-gag) thought to code for the core antigens including the p25, p18 and p13 proteins is located between nucleotidic position 236 (starting with 5' CTA GCG GAG 3') and
 5 nucleotidic position 1759 (ending by CTCG TCA CAA 3'). The structure of the peptides or proteins encoded by parts of said ORF is deemed to be that corresponding to phase 2.

The methionine aminoacid "M" coded by the ATG at position 260-262 is the probable initiation methionine of
 10 the gag protein precursor. The end of ORF-gag and accordingly of gag protein appears to be located at position 1759.

The beginning of p25 protein, thought to start by a P-I-V-Q-N-I-Q-G-Q-M-V-H aminoacid sequence is
 15 thought to be coded for by the nucleotidic sequence CCTATA..., starting at position 656.

Hydrophilic peptides in the gag open reading frame are identified hereafter. They are defined starting from aminoacid 1 = Met (M) coded by the ATG starting from 260-2
 20 in the LAV DNA sequence.

Those hydrophilic peptides are
 12-32 aminoacids inclusive

	37-46	-	-
	49-79	-	-
25	88-153	-	-
	158-185	-	-
	178-188	-	-
	200-220	-	-
	226-234	-	-
30	239-264	-	-
	288-331	-	-
	352-381	-	-
	377-390	-	-
	399-432	-	-
35	437-484	-	-
	492-498	-	-

The invention also relates to any combination of these peptides.

2) The "pol gene" (or ORF-pol)

Figs. 4-12 also show that the DNA fragments
 5 extending from nucleotidic position 1555 (starting with
 5' TTT TTT 3' to nucleotidic position 5086 is thought
 to correspond to the pol gene. The polypeptidic structure
 of the corresponding polypeptides is deemed to be that
 corresponding to phase 1. It stops at position 4583 (and
 10 by 5' G GAT GAG GAT 3').

These genes are thought to code for the virus
 polymerase or reverse transcriptase.

3) The envelope gene (or ORF-env)

The DNA sequence thought to code for envelope
 15 proteins is thought to extend from nucleotidic position
 5670 (starting with 5' AAA GAG GAG A.... 3') up to nucleo-
 tidic position 8132 (ending by A ACT AAA GAA 3').
 Polypeptidic structures of sequences of the envelope
 protein correspond to those read according to the "phase
 20 3" reading phase.

The start of env transcription is thought to be at
 the level of the ATG codon at positions 5691-5693.

Additional feature of the envelope protein coded
 by the env genes appear on figs. 13-18. These are to be
 25 considered as paired figs. 13 and 14 ; 15 and 16 ; 17 and
 18 respectively.

It is to be mentioned that because of format
 difficulties.

Fig. 14 overlaps to some extent with fig. 13.

30 Fig. 16 overlaps to some extent with fig. 15.

Fig. 18 overlaps to some extent with fig. 17.

Thus for instance figs. 13 and 14 must be con-
 sidered together. Particularly the sequence shown on the
 first line on the top of fig. 13 overlaps with the
 35 sequence shown on the first line on the top of fig. 14. In
 other words the starting of the reading of the successive

sequences of the env gene as represented in figs. 13-18 involves first reading the first line at the top of fig. 13 then proceeding further with the first line of fig. 14. One then returns to the beginning of the second line of fig. 13, then again further proceed with the reading of the second line of page 14, etc... The same observations then apply to the reading of the paired figs. 15 and 16, and paired figs. 17 and 18, respectively.

The locations of neutralizing epitopes are further apparent in figs. 13-18. reference is more particularly made to the boxed groups of three letters included in the aminoacid sequences of the envelope proteins (reading phase 3) which can be designated generally by the formula N-X-S or N-X-T, wherein X is any other possible aminoacid. Thus the initial protein product of the env gene in a glycoprotein of molecular weight in excess of 91,000. These groups are deemed to generally carry glycosylated groups. These N-X-S and N-X-T groups with attached glycosylated groups form together hydrophylic regions of the protein and are deemed to be located at the periphery of and to be exposed outwardly with respect to the normal conformation of the proteins. Consequently they are considered as being epitopes which can efficiently be brought into play in vaccine compositions.

The invention thus concerns with more particularity peptide sequences included in the env-proteins and excizable therefrom (or having the same aminoacid structure), having sizes not exceeding 200 aminoacids.

Preferred peptides of this invention (referred to hereafter as a, b, c, d, e, f) are deemed to correspond to those encoded by the nucleotide sequences which extend respectively between the following positions :

- a) from about 8095 to about 8200 ✓
- b) " " 8260 " " 8310 ✓
- 35 c) " " 8390 " " 8440 ✓
- d) " " 8485 " " 8620 ✓

e) - - 6860 - - 5930 ✓
 f) - - 7535 - - 7630 ✓

Other hydrophilic peptides in the env open reading frame are identified hereafter. they are defined starting from
 5 aminoacid 1 = lysine (K) coded by the AAA at position 5670-2 in the LAV DNA sequence.

These hydrophilic peptides are

8-23 aminoacids inclusive

18	63-78	-	-
	82-90	-	-
	97-123	-	-
	127-183	-	-
	197-201	-	-
15	239-294	-	-
	300-327	-	-
	334-381	-	-
	397-424	-	-
	468-500	-	-
20	510-523	-	-
	551-577	-	-
	594-603	-	-
	621-630	-	-
	657-679	-	-
25	719-758	-	-
	780-803	-	-

The invention also relates to any combination of these peptides.

4) The other ORF

30 The invention further concerns DNA sequences which provide open reading frames defined as ORF-Q, ORF-R and as "1", "2", "3", "4", "5", the relative position of which appears more particularly in figs. 2 and 3.

These ORFs have the following locations :

35	ORF-Q	phase 1	start 4478	stop 5086
	ORF-R	" 2	" 8249	" 8896

ORF-1	-	1	-	5029	-	5316
ORF-2	-	2	-	5273	-	5515
ORF-3	-	1	-	5383	-	5616
ORF-4	-	2	-	5519	-	5773
5 ORF-5	-	1	-	7966	-	8279

The LTR (long terminal repeats) can be defined as lying between position 8560 and position 160 (end extending over position 9097/1). As a matter of fact the end of the genome is at 9097 and, because of the LTR structure of the retrovirus, links up with the beginning of the sequence :

Hind III
CTCAATAAAGCTTGCCTTG

15

9097 1

The invention concerns more particularly all the DNA fragments which have been more specifically referred to hereabove and which correspond to open reading frames. It will be understood that the man skilled in the art will be able to obtain them all, for instance by cleaving an entire DNA corresponding to the complete genome of a LAV species, such as by cleavage by a partial or complete digestion thereof with a suitable restriction enzyme and by the subsequent recovery of the relevant fragments. The different DNAs disclosed in the earlier mentioned British Application can be resorted to also as a source of suitable fragments. The techniques disclosed hereabove for the isolation of the fragments which were then included in the plasmids referred to hereabove and which were then used for the DNA sequencing can be used.

Of course other methods can be used. Some of them have been exemplified in the earlier British Application. reference is for instance made to the following methods.

a) DNA can be transfected into mammalian cells with appropriate selection markers by a variety of techniques, calcium phosphate precipitation, polyethylene

glycol, protoplast-fusion, etc..

b) DNA fragments corresponding to genes can be cloned into expression vectors for *E. coli*, yeast- or mammalian cells and the resultant proteins purified.

5 c) The proviral DNA can be "shot-gunned" (fragmented) into procaryotic expression vectors to generate fusion polypeptides. Recombinant producing antigenically competent fusion proteins can be identified by simply screening the recombinants with antibodies against LAV
10 antigens.

The invention also relates more specifically to cloned probes which can be made starting from any DNA fragment according to this invention, thus to recombinant DNAs containing such fragments, particularly any plasmids
15 amplifiable in procaryotic or eucaryotic cells and carrying said fragments.

Using the cloned DNA fragments as a molecular hybridization probe - either by marking with radionucleotides or with fluorescent reagents - LAV virion RNA may be
20 detected directly in the blood, body fluids and blood products (e.g. of the antihemophylic factors such as Factor VIII concentrates) and vaccines, i.e. hepatitis B vaccine. It has already been shown that whole virus can be detected in culture supernatants of LAV producing cells. A
25 suitable method for achieving that detection comprises immobilizing virus onto said a support e.g. nitrocellulose filters, etc., disrupting the virion and hybridizing with labelled (radiolabelled or "cold" fluorescent- or enzyme-labelled) probes. Such an approach has already been
30 developed for Hepatitis B virus in peripheral blood (according to SCOTTO J. et al. Hepatology (1983), 3, 379-384).

Probes according to the invention can also be used for rapid screening of genomic DNA derived from the tissue
35 of patients with LAV related symptoms, to see if the proviral DNA or RNA is present in host tissue and other

tissues.

A method which can be used for such screening comprise the following steps : extraction of DNA from tissue, restriction enzyme cleavage of said DNA, electrophoresis of the fragments and Southern blotting of genomic DNA from tissues, subsequent hybridization with labelled cloned LAV proviral DNA. Hybridization in situ can also be used.

Lymphatic fluids and tissues and other non-lymphatic tissues of humans, primates and other mammalian species can also be screened to see if other evolutionary related retrovirus exist. The methods referred to hereabove can be used, although hybridization and washings would be done under non stringent conditions.

The DNA according to the invention can be used also for achieving the expression of LAV viral antigens for diagnostic purposes.

The invention also relates to the polypeptides themselves which can be expressed by the different DNAs of the inventions, particularly by the ORFs or fragments thereof, in appropriate hosts, particularly procaryotic or eucaryotic hosts, after transformation thereof with a suitable vector previously modified by the corresponding DNAs.

These polypeptides can be used as diagnostic tools, particularly for the detection of antibodies in biological media, particularly in sera or tissues of persons afflicted with pre-AIDS or AIDS, or simply carrying antibodies in the absence of any apparent disorders. Conversely the different peptides according to this invention can be used themselves for the production of antibodies, preferably monoclonal antibodies specific of the different peptides respectively. For the production of hybridomas secreting said monoclonal antibodies conventional production and screening methods are used. These monoclonal antibodies, which themselves are part of

the invention then provide very useful tools for the identification and even determination of relative proportions of the different polypeptides or proteins in biological samples, particularly human samples containing
5 LAV or related viruses.

Thus all of the above peptides can be used in diagnostics as sources of immunogens or antigens free of viral particles, produced using non-permissive systems, and thus of little or no biohazard risk.

10 The invention further relates to the hosts (proca-ryotic or eucaryotic cells) which are transformed by the above mentioned recombinants and which are capable of expressing said DNA fragments.

Finally it also relates to vaccine compositions
15 whose active principle is to be constituted by any of the expressed antigens, i.e. whole antigens, fusion polypep-tides or oligopeptides in association with a suitable pharmaceutical or physiologically acceptable carrier.

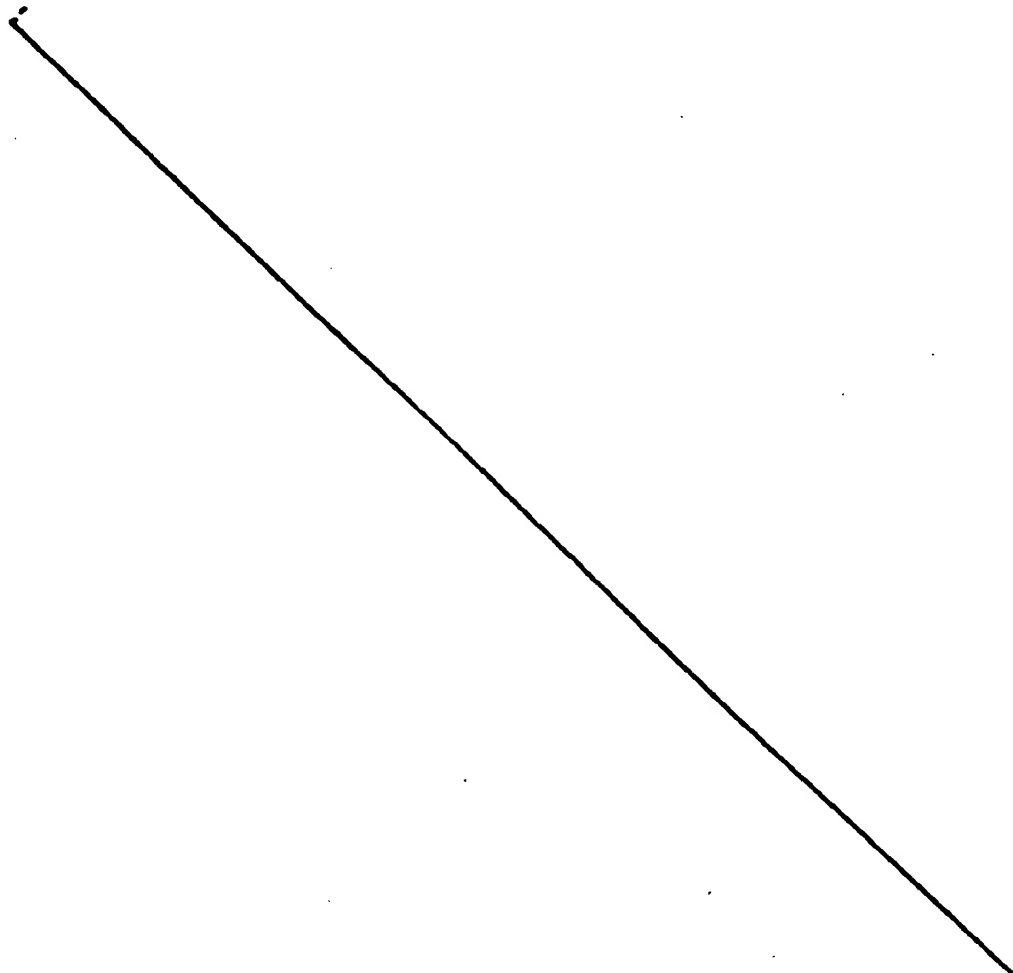
Preferably the active principles to be considered
20 in that field consist of the peptides containing less than 250 aminoacid units, preferably less than 150 as deducible for the complete genomes of LAV, and even more preferably those peptides which contain one or more groups selected from N-X-S and N-X-T as defined above. Preferred peptides
25 for use in the production of vaccinating principles are peptides (a) to (f) as defined above. By way of example having no limitative character, there may be mentioned that suitable dosages of the vaccine compositions are those which enable administration to the host,
30 particularly human host ranging from 10 to 500 micrograms per kg, for instance 50 to 100 micrograms per kg.

For the purpose of clarity figs. 19 to 26 are added. reference may be made thereto in case of difficul-ties of reading blurred parts of figs. 4 to 12.

Needless to say that figs. 19-25 are merely a reiteration of the whole DNA sequence of the LAV genome.

Finally the invention also concerns vectors for the transformation of eucaryotic cells of human origin, particularly lymphocytes, the polymerases of which are capable of recognizing the LTRs of LAV. Particularly said vectors are characterized by the presence of a LAV LTR therein, said LTR being then active as a promoter enabling the efficient transcription and translation in a suitable host of the above defined, of a DNA insert coding for a determined protein placed under its controls.

Needless to say that the invention extends to all variants of genomes and corresponding DNA fragments (ORFs) having substantially equivalent properties, all of said genomes belonging to retroviruses which can be considered as equivalents of LAV.



CLAIMS

1. A DNA fragment of LAV extending from nucleotide position 236 to nucleotide position 1759.

2. A DNA fragment of LAV extending from nucleotide position 1535 to nucleotide position 5086.

3. A DNA fragment of LAV extending from nucleotide position 5670 to nucleotide position 8132.

4. A vector containing a DNA fragment according to any of claims 1 to 3.

5. Peptide corresponding to any of those encoded by the nucleotide sequences which extend respectively between the following positions :

a) from about 6095 to about 6200.

b) " " 6260 " " 6310.

c) " " 6390 " " 6440.

d) " " 6485 " " 6520.

e) " " 6860 " " 6930.

f) " " 7535 " " 7630.

6. Peptide characterized by a sequence of amino-acids deducible from LAV DNA the terminal aminoacids of which extend between the following positions with respect to the lysine (position 1) coded by the AAA at position 5670-5672 in the LAV DNA.

8-23 aminoacids inclusive

63-78 " "

82-90 " "

97-123 " "

127-163 " "

197-201 " "

239-294 " "

300-327 " "

334-361 " "

397-424 " "

466-500 " "

510-523 " "

551-577 " "

594-803 " "
 821-830 " "
 857-878 " "
 719-758 " "
 5 780-803 " "

or any combination of these peptides.

7. Peptide corresponding to the aminoacid sequences deducible from LAV DNA and the terminal aminoacids of which are positionned at the positions hereafter counted from the Met at position 1 coded by the ATG sequence at nucleotide positions 280-2 :

12-32 aminoacids inclusive

	37-48	"	"
	49-79	"	"
15	88-153	"	"
	158-165	"	"
	178-188	"	"
	200-220	"	"
	228-234	"	"
20	239-264	"	"
	288-331	"	"
	352-381	"	"
	377-390	"	"
	399-432	"	"
25	437-484	"	"
	482-498	"	"

and combination of said peptides.

8. Diagnostic means containing any of the DNA fragments of any of claims 1 to 3.

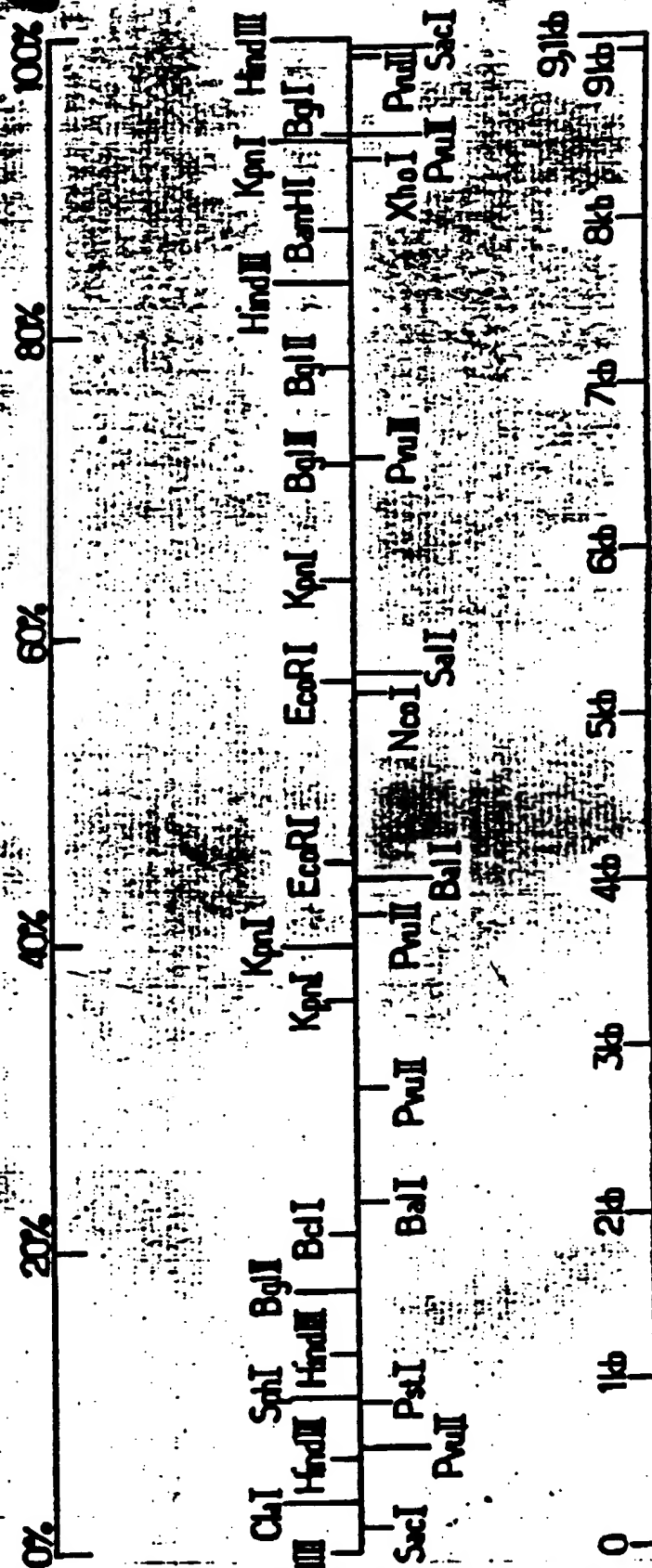
30 9. Diagnostic means containing any of the peptides of any of claims 4 to 8.

10. Vaccine compositions containing any of the peptides according to any of claims 4 to 8 in association with a pharmaceutical vehicle.

35

End of transmission

FILE



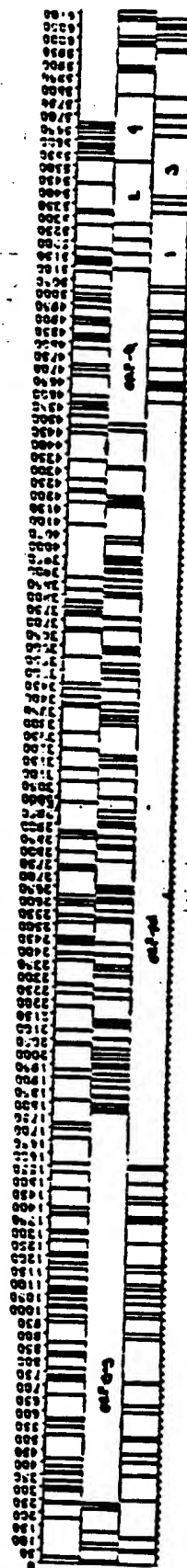


Fig. 2

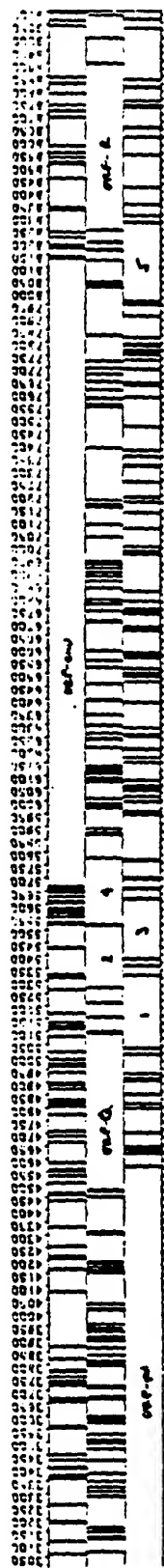


Fig. 3

K 9 V 0 P V J M S G H K I 2 I K M I L 0 D L C P V L 0 M S 4 S H A S I I C C K
 a 0 Y S I L U I 0 J L P K F B J V V C K F V M I L Q A F O A S O I V A
 I C I A L P S A F W I 0 M D U K M P I L I C S I M L 0 P S K L M B 0 0 V 4
 A A G A I G T A G C C I D C A G A T A G C A G A C C C T T A G A C A T A T G A C C C T C I A A A A C T C I A A A C C T C A C C A C C T C A A
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M L D H V L V G P M C E P N L O D V F N S I G I S S V I R O M D S Y S G S C
 M A I E T L L V O M A 4 P U C K T I L K A L C P A A T L E E P M T A C O C O C V C
 I G O J M P C A S M N H T Q I V R L F O K M W D G Q L H M K K O O M H V R E L E
 A A T T G C A C A C A A C C T T G T G C T C A A A T G C T A R C C C A G A T T G A N G A C T A T T T T A A A C A T T G C A C C G C C F C A C A T A G A C A A A T A T A C A C A C A C A C G C G C G C
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[illegible][illegible]

4 E D L A F L D G A 2 F F S S E J T R A 4 S P I 2 0 E L O V 6 R D N M Y L S
C A I A P S V M C N P C V I L : S V P E P I A P P E S F A S C V E I T I P S O
C G L P T O E C G C I F F A D O S I A O P W C K R A S C L C O R O L P L A
A C G G A G A T C C C T T E C T T A C A G C C A C C A G A C C A G A C C C C C C C A C C A G A C C T T C A G C C T C C C T C C T C A
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680

E A G A D R Q G T V S F N F P O I T L A O B P L V I K I G C G L K E A L L O T
K U F P I D N F L V P L T S L F S L F C V D P S S O P O C C A T O R K L V O I O
S Q S A Q V R V C I L L P S D H S L A T I P R H M K D P C A T K G S I R V N
G A G C A G C C G A T A G C G A C T U T T A C T C C A G C A C T T G C A G C C C T C A C A A G A T A G A G G G G C A C T A A G A G A C T C T A T A G C T A C A
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6 A C A I A V L E I A S L P C M A P A I C I C I A G I M V A O V O I L I E I
I C A I O V A A M A C J I D C I O K O C I E V L S M O Q S V I R V S O M S
S C A S I I A A I A P A E I A A D O C M W F V Q S K I V O S O I H R M L
S C C A G A I C A C A I A C A I I C C C A G A C A C A A A T C I A C C C A A I T T C A A G I A A C A L A C I A A C A T A C A C A A C
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020

Year	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
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about on F. 101

[illegible]

Nov 1967

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O U R J C B K O P 3 H A O O V G F L L V L H V P V K E M E Y H C E S I C H A S
V A C S A A U M A H S W P C D S I O I T M I Y C M W C Y P V D Q V L A S
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[illegible][illegible][illegible]

" E A V L A d v p A + H M L I C G Y F U V D = L V S A C I R N W L F L D C I O N A
 P A R S I J U L U I F A F L F P Y K O I Y O S V L F S C Q V Y F + O f + i A
 G A C L S G T S Y W M R O V S R O I S H C V O E S I F P O H A O G
 AAGCAGGCTTAETCCCATGGACATCAACAAACGAATTGCACCAATTCAGTAAATTTAGTCAGCTCGAATCGGAGAGACTATTTTATGATGATATAAG
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[illegible]

Year	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
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[illegible]

[illegible]

Fig. 6

[illegible]

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Fig 11

Start ORP-R

600-2
 C C A W V N K O C G N A V C K C K Y F I S O A S S R A C C S I A R P C M T
 V A S C O W V A L C W P L V B E K R R A F P A A O C V C A S R O D L F K H C
 66GTGGCAATGTCACAAAGTACTGCTGCGATCCCTACTGTAAGCGAGAAATATGACAGCTACCGACACATCGGCGCCAGCCAGCATCTCCAGACCCGCAAAACATCG
 8290 4300 4300 4310 4320 4330 4340 4350 4360 4370 4380 4390 4400

1 N H K O U V S S V O C C L C L A R S T O G L C C C F S M T S C T F K T M D L V
 2 A I I S M T A A T M A C A W L F A O F E F C V C F P V I P C V P L R M D L V
 3 O S O V A I U C L P P C C K M R R W F C S M L R V L R D O O L I
 A C C A I C A C A G T A G C A I C A C C C T T C T T C T T C T T C A A C A A G C A C C C A C C C T T T C A G T C A C C C T A C C T T T A G A C C C A A G C A C T T A
 8410 1420 1430 8440 8450 8460 8470 8480 8490 8500 8510 8520

U S C S R O P T S N I S T A I N A I S I A H T

K A A V U L S H F L M E K C C A E C L I H S O P E R O D I L D L M I V M I O C V F
 R Q L O I L A T F O K K R C D M K C O F I P M E D M I S L I C G S Y T M A T S
 CAAGCAGCTGTAGACTTACCCATTTTAAAGAAAGCCGACCTGGACGGCTAATTCATCTCCACGACACATATCTTCGAGTCTACCCACACAGGCTCTT
 8530 8540 8550 8560 8570 8580 8590 8600 8610 8620 8630 8640

P ° L A E L M T R A B C O I S T D L M N V L O A S T S ° A B ° C R R C D ° R P F E
 L D M Q N V I T P C P C V A N P L T F C M C V K L V P V E P D K V E E A M K C E M
 C C C T G A T T C C A G A C A C C G C C C A G C A T A T C C A C T T T G C T G C T A A G C T A G C A C T T G A C C C A T A A G C A C A A
 8650 8660 8670 8680 8690 8700 8710 8720 8730 8740 8750

M 3 L V I P C E F A A H C O P O E R S V R V F V O Q P P S I S S R C P R A A S C
 T S L L W P V S L M G M U O F R E V L E A B F D S R L A F M H V A R E L M P F
 P A C V I A C N I L R F M C O S C G L T A A M F I T W P E S C I R S
 C A C C G C T T G A C C T C G A C C A T C A C C T C A G A G A A C T T T A C G C A G C T T A C C C C C T A C C T C C C C G A C C T C A C C C A
 8770 8780 8790 8800 8810 8820 8830 8840 8850 8860 8870 8880

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